

DTIC  
ELECTE  
S JAN 22 1992 D

92-01523



# Effects of Granulocyte-Macrophage Colony-Stimulating Factor in Burn Patients

MAJ William G. Cioffi, Jr, MC, USA; LTC David G. Burleson, PhD; Bryan S. Jordan, MS; LTC William K. Becker, MC, USA;  
COL William F. McManus, MC, USA; Arthur D. Mason, Jr, MD; COL Basil A. Pruitt, Jr, MC, USA

• We studied the effects of granulocyte-macrophage colony-stimulating factor in burn patients. Serial measurements of granulocyte oxidative function were obtained in treated patients and in a group of controls matched for age and total burn size. The administration of granulocyte-macrophage colony-stimulating factor resulted in a 50% increase in mean leukocyte counts. Both groups showed significant baseline increases in granulocytic cytosolic oxidative function. Treated patients showed normal stimulated cytosolic oxidative function, which was significantly depressed compared with that of untreated patients. Myeloperoxidase activity was increased in treated patients during the first postburn week but then declined to normal levels. Untreated patients had a significant increase in myeloperoxidase activity for the first 3 weeks following injury. Untreated patients exhibited a significant decrease in superoxide activity during the second 3 weeks following injury. Treated patients demonstrated normal superoxide activity.

(Arch Surg. 1991;126:74-79)

Improvements in fluid management, wound care, and nutritional support have markedly reduced early mortality from thermal injury, but significant late mortality persists. Burn-induced defects of the immune system appear to contribute to late mortality, which is primarily due to infection and sepsis.

Although the specific cause of the immune dysfunction following thermal injury is unknown, both qualitative and quantitative defects have been noted in all limbs of the immune system.<sup>1-13</sup> Defective migration, phagocytosis, and degranulation have been described as manifestations of granulocyte dysfunction. In addition, burn serum contains an inhibitor of complement conversion that may cause opsonization failure that further inhibits neutrophil function.<sup>14</sup> Such

granulocyte dysfunction may contribute significantly to the marked predisposition to infection.<sup>15</sup>

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a lymphokine that was first described nearly 20 years ago. Not only does GM-CSF stimulate the proliferative potential of granulocyte and macrophage progenitor cells in the bone marrow, but it also stimulates various functional activities of mature cells. In the presence of GM-CSF, macrophages are stimulated to secrete plasminogen activating factor<sup>16</sup> and also exhibit increased phagocytic and cytotoxic activity for bacteria, yeast,<sup>17</sup> and malignant cell lines.<sup>18</sup> Granulocytes increase RNA and protein synthesis and exhibit increased antibody-dependent cytotoxic killing of tumor cells and enhanced oxidative metabolism in the presence of GM-CSF in vitro.<sup>17,19-22</sup> Recombinant GM-CSF stimulates mature neutrophils to augment cell surface antigenic expression as well as increase their phagocytic activity, synthesis of biologically active molecules, and expression of various cell surface markers.<sup>23</sup>

In a group of patients with thermal injury, a comparison of the serum levels of hematopoietic colony-stimulating factors (CSFs) has revealed distinct differences between survivors and nonsurvivors.<sup>24</sup> The nonsurviving patients demonstrated an inappropriate lag in the generation of CSF early in the course of burn injury and inappropriately low levels of the factor even in the presence of documented sepsis. This aberrant response was associated with a relative failure of granulopoiesis. Further studies have demonstrated that serum from patients with thermal injury inhibits the in vitro production of CSFs by mononuclear cells.<sup>25</sup>

The multiple defects in granulocytic function and the decreased levels of CSFs following lethal thermal injury suggest that a beneficial effect on granulocyte count and function might result from the administration of CSFs. Our study was designed to determine the safety of the administration of human recombinant GM-CSF (hr-GM-CSF) in patients with thermal injury.

## PATIENTS AND METHODS

### Patient Population

Patients with burns over 20% to 70% of the total body surface area were eligible for enrollment in the study. Patients with inhalation injury diagnosed by xenon 133 lung scanning but with no broncho-

Accepted for publication September 29, 1990.

From the US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Tex.

Read before the Tenth Anniversary Meeting of the Surgical Infection Society, Cincinnati, Ohio, June 15, 1990.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Reprint requests to the US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, TX 78234-5012 (Dr Pruitt).

scopic evidence of upper airway injury were also eligible for enrollment. Bronchoscopic evidence of inhalation injury resulted in exclusion from the study. All eligible patients were admitted within 48 hours of injury and underwent uneventful resuscitation. Routine care was not altered. In all patients, sulfadiazine silver was applied once daily. Patients treated with other lymphokines, prophylactic antibiotics, or corticosteroids were excluded from the study. Data from patients with thermal injury admitted during the same period but not enrolled in the study and a group of healthy laboratory controls were obtained for comparison with the treated patients.

### hr-GM-CSF

Nonglycosylated hr-GM-CSF was obtained from the bacterial fermentation of a strain of *Escherichia coli* bearing a genetically engineered plasmid containing the human GM-CSF gene. The product is a highly purified, sterile, stable, water-soluble protein with a molecular weight of 14 477 d. The GM-CSF was shown to be biologically active in the KG-1 cell proliferation assay and a colony-stimulating assay that employed bone marrow cells.

### Drug Administration

Patients were administered 3 or 10  $\mu\text{g/kg}$  daily of hr-GM-CSF intravenously during a 4-hour period. Treatment began within 5 days of injury and continued for a minimum of 2 weeks or until a grade 3 or 4 toxic reaction developed. All potential adverse effects were recorded and graded on the following scale: 1, mild; 2, moderate; 3, severe; and 4, life-threatening. Any patient who experienced a grade 3 or 4 toxic reaction that was deemed attributable to the hr-GM-CSF received no further treatment until the adverse reaction resolved. The patient was then re-treated at a dosage not more than 50% of the original. Recurrence of the same toxic reaction necessitated withdrawal from the study. In patients who exhibited a white blood cell count greater than  $50.0 \times 10^9/\text{L}$ , subsequent doses of the lymphokine were withheld until the white blood cell count decreased to less than  $30.0 \times 10^9/\text{L}$ . Administration was then resumed at a dose of 30% to 50% of the original dose.

### In Vitro Testing

Complete blood cell counts were obtained daily from each patient. In vitro granulocyte function tests were performed twice weekly during treatment and for up to 3 weeks following cessation of lymphokine administration. Granulocytes were isolated from heparinized whole blood by Ficoll-Hypaque gradients. Cells passing through the gradient were recovered from the cell pellet. Contaminating red blood cells were removed by hypotonic lysis. The cell pellet from the Ficoll-Hypaque gradient was resuspended in 50 mL of Hanks' balanced salt solution (HBSS), spun at 2250g for 10 minutes, and 3 mL of the buffy coat was removed and placed in a 50-mL conical centrifuge tube. Distilled water (20 mL) was added during agitation of the sample on vortex mixer. After 20 seconds, 20 mL of hypertonic ( $2 \times$ ) HBSS was added, the cells were centrifuged at 200g for 10 minutes, and the supernatant was removed. The cells were suspended in 2 mL of HBSS and transferred to a 15-mL conical centrifuge tube. A second lysis was performed with the addition of 4 mL of distilled water for 20 seconds, after which 4 mL of  $2 \times$  hypertonic HBSS was added to restore isotonicity. The cells were suspended at a concentration of  $1 \times 10^6$  cells/mL in 1 mL of barbital buffer (pH 7.25).<sup>26</sup> 2',7'-Dichlorofluorescein diacetate (DCF-DA, at a final concentration of 5  $\mu\text{mol/L}$ ) was added to each sample and incubated for 20 minutes at 37°C to allow DCF-DA to enter the cells. Whereas DCF-DA easily permeates the cells where the acetyl groups are hydrolyzed to 2',7'-dichlorofluorescein (DCF), the DCF is too polar to pass through the plasma membrane and is effectively trapped within the cell. When oxidized by peroxide, DCF becomes highly fluorescent and the measurement of this fluorescence serves as an index of cytosolic peroxidative activity. Cell fluorescence was measured by flow cytometry. The mean fluorescence of 10 000 cells was calculated for each data point. After an initial fluorescent measurement, cells were incubated for 20 minutes with and without phorbol myristate acetate (PMA, 700 ng/mL) as stimulant. Measurements were recorded as log fluorescence and were compared with values obtained from granulocytes from healthy volunteers.

Additional studies of granulocyte oxidative metabolism were performed with the use of two chemiluminescent probes, luminol, and dimethyl biacridinium dinitrate (DBA).<sup>25</sup> Heparinized whole blood

was diluted 1:10 in HBSS (pH 7.2). Aliquots (20  $\mu\text{L}$ ) of diluted whole blood were added to 2 mL of barbital buffer solution in siliconized glass vials. The appropriate chemiluminescent probe was then added to each sample, and three prestimulation background measurements were performed. All measurements were made at 25°C in a liquid scintillation counter set for photon counting. Saline, PMA (350 nM/L), or zymosan (6.25 mg/L), preopsonified with guinea pig serum, was added to the vial, and luminescence was measured at 13-minute intervals for 2 hours. The total luminescence produced in each sample was calculated from the light-intensity measurements by trapezoidal approximation. The values obtained for luminol correspond to the total oxygenation events produced primarily by myeloperoxidase. The values obtained when DBA was used as a probe corresponded to the total oxygenation events produced by extracellular superoxide anion and other oxidative species.

### Statistical Analysis

Differences between groups were analyzed with use of the *t* test and analysis of variance, with post hoc testing, when appropriate, with use of the BMDP statistical package.

## RESULTS

### Patient Population

Ten patients with a mean age of 28.6 years and a mean burn size of 37% were enrolled in the study. Individual patient data, including the dose of hr-GM-CSF, and the duration of treatment, are outlined in Table 1. Two patients, both with inhalation injury, died, for a mortality rate of 20%. Fourteen patients with thermal injury with a mean age of 30.5 years and mean burn size of 36%, admitted during the same period, were used as nonrandomized controls for comparison of oxidative metabolism data. There was no statistical difference between the two groups of patients with respect to age, burn size, and mortality rate, although a greater proportion of untreated patients had inhalation injury (Table 2). Grades 1 and 2 adverse effects were common. Seven patients complained of pruritus, four exhibited pyrexia during administration of hr-GM-CSF, two complained of back pain, and one experienced pleuritic chest pain. Acute parotitis and a subcutaneous abscess occurred in one patient, each requiring incision and drainage.

### Blood Count Data

Patients receiving GM-CSF demonstrated a significant increase in total white blood cell count during the second postburn week compared with the first, third, fourth, fifth, sixth, and seventh postburn weeks (Fig 1). One patient, who received 10  $\mu\text{g/kg}$  of GM-CSF, had a white blood cell count greater than  $50.0 \times 10^9/\text{L}$  during the second postburn week. Lymphokine administration was discontinued for 2 days, during which time the white blood cell count decreased to  $26.0 \times 10^9/\text{L}$ , and treatment was then resumed at 3  $\mu\text{g/kg}$  per day. The majority of treated patients demonstrated a relative decrease in their white blood cell counts during the third postburn week despite continued administration of GM-CSF. Compared with the untreated burn patients, the patients receiving GM-CSF exhibited a significant elevation in their white blood cell counts only during the second postburn week. The percentage of granulocytes was not different between treated and untreated burn patients during the first 3 weeks. However, on cessation of GM-CSF administration, a significant decrease in the percentage of granulocytes was noted in the treated patients compared with the untreated burn patients (63.5% vs 80.9%) (Fig 2). The percentage of polymorphonuclear cells was not different during treatment but decreased significantly during the fourth postburn week compared with untreated patients (45.3% vs 71.2%), accounting for the difference in the granulocyte percentages (Fig 2). No statistically significant differences between treated and untreated patients were noted in the percentage of mono-

Table 1.—Patient Demographics\*

Patient/ Age, y/Sex	% Total Burn Surface Area	% Full- Thickness	Granulocyte- Macrophage Colony- Stimulating Factor Dose, $\mu\text{g/kg}$	Duration of Treatment, d
1/24/M	36	32	3	17
2/45/M	39	5	3	17
3/24/M	24	18	10	17
4/27/M	20	17	10->3	12
5/24/M	35	0	3	12
6/35/F	23	23	3	11
7/23/M	45	42	3	17
8/22/M	54	44	3	5
9/21/F	42	8	3	29
10/41/M	52	10	3	2

\*Factor administration was stopped on days 5 and 2 in patients 8 and 10, respectively, because of worsening pulmonary status. Both patients had abnormal xenon 133 bone scans but normal bronchoscopic findings. The degradation in pulmonary function was not temporally related to the administration of factor.

Table 2.—Comparison of Treated and Untreated Groups\*

	Treated	Untreated
Age, y	28.6 $\pm$ 2.7	30.6 $\pm$ 3.0
% total burn surface area	37.1 $\pm$ 3.8	36.2 $\pm$ 3.0
Inhalation injury	2/10 (20)	6/14 (43)
Mortality	2/10 (20)	0/14 (0)

\*Values are mean  $\pm$  SD or number affected/total number (percent). No difference in age or percent total burn surface area was noted between the two patient groups, but the untreated patients had a higher incidence of inhalation injury.

cytes, lymphocytes, myelocytes, or band forms either during or after treatment, although patients receiving the cytokine tended to have an increased percentage of band forms and myelocytes during treatment.

#### Dichlorofluorescein Oxidation

No significant difference in baseline unstimulated cytosolic oxidative activity was noted between the two patient groups, although both were significantly higher than values for unburned controls (Table 3). Patients receiving hr-GM-CSF exhibited a significant decrease in maximal cytosolic oxidative activity compared with untreated burn patients (92.9% vs 114.7% of control values;  $P < .01$ ) during the 3 weeks of treatment. On cessation of cytokine administration, the peak cytosolic oxidative activity of treated patients increased slightly to 97% of that for controls whereas untreated patients remained elevated at 113% of control values (Table 4).

#### Chemiluminescence

During the first 7 days following injury, both treated and untreated burn patients exhibited a significant increase in luminol chemiluminescence compared with healthy controls. This increase was independent of the type of stimulation employed to activate the granulocytes, as the response to opsonified zymosan and PMA were essentially identical (Table 5). After 1 week, oxidation of luminol following stimulation by opsonified zymosan and PMA decreased to control values for the treated patients but remained elevated for the untreated patients. During the third postburn week (the last

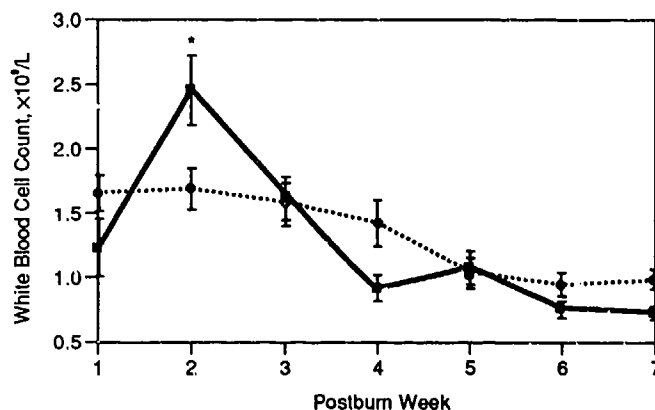


Fig 1.—White blood cell counts during the first 7 postburn weeks are shown for the treated (solid line) and untreated (broken line) patients. The only significant difference was detected during the second postburn week (asterisk equals  $P < .05$ ). Vertical bars indicate SDs.

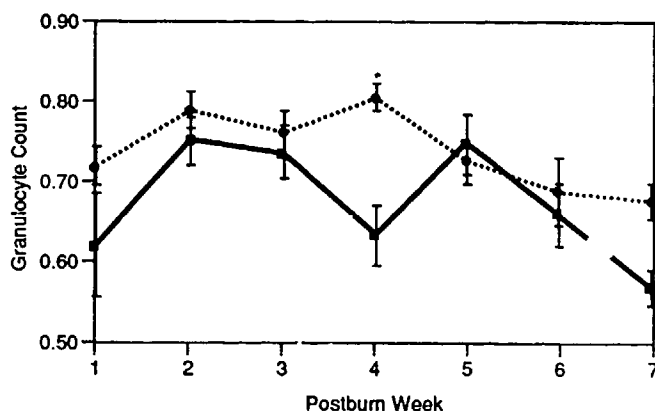


Fig 2.—The percentages of granulocytes for treated (solid line) and untreated (broken line) patients are displayed for the first 7 postburn weeks. No differences between groups were evident, except during postburn week 4 when treated patients had significantly fewer granulocytes than untreated patients. Asterisk equals  $P < .05$ . The percentage of lymphocytes increased in treated patients, but the difference between groups was not significant. Vertical bars indicate SDs.

week of therapy with GM-CSF), luminol chemiluminescence remained unchanged in the untreated patients and increased significantly in the treated patients in response to PMA but not opsonified zymosan administration. After 21 days, luminol chemiluminescence began to decrease in untreated patients but remained significantly elevated in treated patients in response to PMA administration.

The oxidation of DBA, an indicator of extracellular superoxide production, normally declines as time after injury progresses. In this group of untreated burn patients, the mean luminescence value was 87% of the control patients' mean value during the first 3 weeks and 61% of the control value ( $P < .01$ ) during the second 3-week period following injury (Table 5). Patients treated with GM-CSF failed to show a decrement in oxidation of DBA, with luminescence values similar to those for controls during the first 2 weeks following injury and 137% of those for controls during the third and final week of treatment. The DBA chemiluminescence remained significantly elevated at 117% of control values when administration of GM-CSF was discontinued.

## COMMENT

Adequate numbers of properly functioning granulocytes may be one of the most important factors in a patient's defense against infection. Thermal injury induces a variety of abnormalities in granulocyte production and function. Peterson et al<sup>27</sup> have reported decreased numbers of circulating granulocyte stem cells in nonsurviving patients with large burns, which was thought to reflect a reduction of the bone marrow progenitor cell pool. This decrease in circulating colony-forming units was associated with a higher incidence of fatal septicemia. Defects in chemotaxis, random migration, phagocytosis, bactericidal capacity, superoxide production, and in vitro oxygen consumption have all been described, but a

relationship between these defects and the propensity for infection has not been shown.

Granulocyte-macrophage CSF is a cytokine produced by activated T cells and macrophages as well as by certain fibroblasts and endothelial cells.<sup>28</sup> It is a potent stimulus of bone marrow progenitor cell production of neutrophils, monocytes, and eosinophils. Significant increases in numbers of circulating granulocytes have been documented in both healthy primates and humans following parenteral administration of GM-CSF.<sup>17,21,24-25,29-31</sup> Clinical trials in patients with leukopenia secondary to aplastic anemia,<sup>32</sup> acquired immunodeficiency syndrome,<sup>33</sup> chronic idiopathic neutropenia,<sup>34</sup> and chemotherapy-induced neutropenia<sup>35-37</sup> have all shown the ability of GM-CSF to increase circulating levels of mature granulocytes. Parenteral administration of GM-CSF to our cohort of patients with thermal injuries resulted in a similar response. After a lag time of approximately 1 week, white blood cell counts increased significantly compared with untreated burn patients. After cessation of GM-CSF administration, counts quickly decreased to expected normal levels. Eosinophilia, commonly seen in primate studies following the parenteral administration of GM-CSF, was not observed in our treated patients.

The in vitro effect of GM-CSF on white blood cells isolated from healthy volunteers has been well documented. Although GM-CSF has little effect on white blood cell function alone, it appears to "prime" the cell for increased oxidative function when activated in vitro by physiologic chemoattractants, such as PMA, FMLP (F Met-Leu-Phe), C5a, leukotriene B<sub>4</sub>, and opsonified zymosan.<sup>38</sup> Chemotaxis, cytotoxic and phagocytic activity, superoxide production, and degranulation are all increased by prior incubation with GM-CSF.<sup>39</sup>

Few data exist concerning the effect of parenteral GM-CSF on various white blood cell functions in patients with documented functional defects. Defects in granulocyte phagocytosis and bactericidal capacity in two patients with acquired immunodeficiency syndrome were resolved with the parenteral administration of GM-CSF.<sup>40</sup> Reductions in phagocytic capacity, nitroblue tetrazolium reduction, and migration were restored to normal by the administration of GM-CSF in one patient with chronic idiopathic neutropenia.<sup>41</sup>

Table 3.—Cytosolic Peroxidase Activity for Postburn Days 0 Through 21\*

	PTUR	PTSR
Treated	0.249 ± 0.01	0.929 ± 0.06
Untreated	0.243 ± 0.01	1.150 ± 0.05

\*Values are mean ± SD. PTUR indicates the ratio of the mean log fluorescence for unstimulated patients' cells to stimulated control subjects' cells (normal, 0.16) (both patient groups were significantly different from controls ( $P < .05$ )); PTSR, the ratio of the mean log fluorescence for stimulated patients' cells to stimulated control subjects' cells (normal, 1.0) (untreated patients were significantly different from treated patients and controls ( $P < .05$ )).

Table 4.—Cytosolic Peroxidase Activity for Postburn Days 22 Through 42\*

	PTUR	PTSR
Treated	0.223 ± 0.02	0.972 ± 0.05
Untreated	0.227 ± 0.01	1.140 ± 0.04

\*Values are mean ± SD and are for the 3 weeks after cessation of factor administration. See Table 3 for an explanation of PTUR and PTSR. The PTUR for both patient groups remained significantly elevated compared with controls ( $P < .05$ ); PTSR for untreated patients remained significantly different from treated patients and controls ( $P < .05$ ).

Table 5.—Chemiluminescence Data\*

	LOZ	LPMA	DPMA
<b>PBD 0-7</b>			
Control (n = 137)	2344 ± 179	2482 ± 158	14371 ± 944
Untreated (n = 14)	4060 ± 686	6635 ± 784	11076 ± 1803
Treated (n = 11)	6355 ± 3047†	7523 ± 4513†	10932 ± 4956
<b>PBD 8-14</b>			
Control (n = 137)	2344 ± 179	2482 ± 158	14371 ± 944
Untreated (n = 24)	4298 ± 912†	5330 ± 763†‡	12036 ± 1670
Treated (n = 15)	2302 ± 750	2989 ± 861	14248 ± 4934
<b>PBD 15-21</b>			
Control (n = 137)	2344 ± 179	2482 ± 158	14371 ± 944
Untreated (n = 22)	4779 ± 987†	4919 ± 678†	13110 ± 2170
Treated (n = 14)	3649 ± 593	3943 ± 632§	19312 ± 4904
<b>PBD &gt;21</b>			
Control (n = 137)	2344 ± 179	2482 ± 158	14371 ± 944
Untreated (n = 74)	3120 ± 319	4009 ± 897	8781 ± 961†‡
Treated (n = 44)	3217 ± 469	5141 ± 1166§	16791 ± 2422

\*Values are mean ± SD chemiluminescence data. LOZ indicates opsonified zymosan-stimulated luminol chemiluminescence; LPMA, phorbol myristate acetate (PME)-stimulated luminol chemiluminescence; DPMA, PMA-stimulated dimethyl biacridinium dinitrate chemiluminescence; and PBD, postburn day.

†Significant difference compared with controls ( $P < .01$ ).

‡Significant difference compared with treated patients ( $P < .01$ ).

§Significant difference compared with controls ( $P < .05$ ).

The parenteral administration of GM-CSF to our group of patients with thermal injury did not affect the baseline (non-stimulated) increase in *in vitro* cytosolic oxidative activity previously described.<sup>40</sup> When the oxidation of DCF is expressed as a percentage of the mean fluorescence of stimulated white blood cells from healthy control subjects, unstimulated cells from healthy controls demonstrate approximately 16% activity. Both the treated and untreated burn patients' cells had significantly higher baseline activity compared with normal controls (24.9% and 24.3%, respectively). This increase in unstimulated oxidative capacity persisted even after discontinuation of the GM-CSF. Patients receiving GM-CSF had normal stimulated DCF oxidation values (92.9%) that were significantly lower than the 115% activity seen in white blood cells from untreated patients. Thus, it appears that GM-CSF decreases the capacity of granulocytes to oxidize DCF, presumably due to the lower production of intracellular hydrogen peroxide.

Myeloperoxidase activity, as indexed by luminol chemiluminescence following stimulation by opsonified zymosan and PMA, was markedly elevated in untreated patients for the first 3 weeks after injury. Treated patients showed a significant increase in luminol chemiluminescence during the first few days of treatment, which subsequently declined to normal control values during the second week of treatment. During the third week of treatment and on discontinuation of GM-CSF administration, opsonified zymosan-stimulated chemiluminescence remained normal. In contrast, PMA-stimulated luminol chemiluminescence rose to supranormal levels.

The level of DBA chemiluminescence, which primarily indexes superoxide anion production, was significantly affected by the administration of GM-CSF. During the first 3 weeks after injury, granulocytes from untreated patients showed normal to slightly decreased PMA-stimulated chemiluminescence when DBA was used as a probe. During the subsequent 3 weeks, this defect was exaggerated. In patients receiving GM-CSF, DBA chemiluminescence was only slightly depressed during the first postburn week, normal during the

second postburn week, and supranormal during the third week of drug administration. After discontinuation of GM-CSF administration, DBA chemiluminescence remained normal and did not decrease in contrast to that in the untreated patients. The maintenance of DBA chemiluminescence following cessation of GM-CSF administration indicates that the effect of the cytokine is not direct, because the half-life of circulating neutrophils is substantially less than 1 day.

The administration of parenteral GM-CSF to patients with thermal injury but without inhalation injury appears to be safe and resulted in the expected increase in circulating numbers of granulocytes. Whether this compound can be safely administered to patients with inhalation injury cannot be answered from our study. Although both patients with inhalation injury who received GM-CSF died, deterioration in the patients' status was not temporally related to its administration. A more complex question concerns whether the effect of parenteral administration of GM-CSF on white blood cell function is beneficial. Restoration of superoxide production by stimulated cells has the potential for both beneficial and adverse effects. An increase in extracellular superoxide may lead to an increase in capillary permeability due to endothelial injury from adherent white blood cells. The reduction in myeloperoxidase activity might also be viewed as detrimental to the patient, as this enzyme plays an important role in the bactericidal capabilities of the phagocyte. The effect of these changes on morbidity and mortality cannot be determined from our nonrandomized trial of GM-CSF administration in patients with limited thermal injury. Our results caution against the extrapolation of data obtained through the *in vitro* incubation of normal cells with GM-CSF. Future studies concerning the effect of parenteral administration of GM-CSF on white blood cell function in healthy subjects as well as its effect on pulmonary function in lung injury in animal models will be important to define the *in vivo* effects and the potential beneficial or detrimental effects when administered to injured patients.

The hr-GM-CSF used in this study was kindly supplied through a joint effort between Schering-Plough Corp and Sandoz Corp.

## References

1. Miller CL, Baker CC. Changes in lymphocyte activity after thermal injury: the role of suppressor cells. *J Clin Invest*. 1979;63:202-210.
2. Miller L, Trunkey DD. Thermal injury: defects in immune response induction. *J Surg Res*. 1977;22:621-625.
3. Neilan BA, Taddei L, Strate RG. T lymphocyte rosette formation after major burns. *JAMA*. 1977;238:493-496.
4. Ninnemann JL. Immunosuppression following thermal injury through B cell activation of suppressor T cells. *J Trauma*. 1980;20:206-213.
5. Ninnemann JL. Activation of suppressor thymus derived cells following thermal injury is bone marrow derived and not accessory cell macrophage mediated. *Immunol Lett*. 1979;1:97-100.
6. Ninnemann JL, Fisher JC, Wachtel TL. Thermal injury-associated immunosuppression: occurrence and *in vitro* blocking effect of post recovery serum. *J Immunol*. 1979;122:1736-1741.
7. Kohn J, Cort DF. Immunoglobulins in burned patients. *Lancet*. 1969;1:836-837.
8. King RD, Kaiser GC, Lempke RE, Ruster MH. The delayed anamnestic response to tetanus toxoid. *Surg Gynecol Obstet*. 1963;116:745-749.
9. Kay GD. Prolonged survival of a skin homograft in a patient with very extensive burns. *Ann NY Acad Sci*. 1967;64:767-774.
10. Chamberl J, Batchelor JR. Influence of defined incompatibilities and area of burn on skin-homograft survival in burned subjects. *Lancet*. 1969;1:16-18.
11. Ninnemann JL, Fisher JC, Frank HA. Prolonged human allograft rejection due to the spontaneous immunosuppression following thermal injury. *Transplantation*. 1978;25:69-72.
12. Warden GD, Mason AD Jr, Pruitt BA Jr. Evaluation of leukocyte chemotaxis *in vitro* in thermally injured patients. *J Clin Invest*. 1974;54:1001-1004.
13. Davis JM, Dineen P, Gallin JI. Neutrophil degranulation and abnormal chemotaxis after thermal injury. *J Immunol*. 1980;124:1467-1471.
14. Bjornson AB, Altemeier WA, Bjornson HS. Complement, opsonins, and the immune response to bacterial infection in burned patients. *Ann Surg*. 1980;191:323-329.
15. Alexander JW, Ogle CK, Stinnett JD, et al. A sequential, prospective analysis of immunologic abnormalities and infection following severe thermal injury. *Ann Surg*. 1978;188:809-816.
16. Lin HS, Gordon S. Secretion of plasminogen activator by bone marrow-derived mononuclear phagocytes and its enhancement by colony-stimulating factor. *J Exp Med*. 1979;150:231-235.
17. Handman E, Burgess AW. Stimulation of granulocyte-macrophage colony-stimulating factor of *Leishmania tropica* killing by macrophages. *J Immunol*. 1979;122:1134-1137.
18. Grabstein KH, Urdal DL, Tushinski RJ, et al. Induction by macrophage tumor-inhibitory activity by granulocyte-macrophage colony-stimulating factor. *Science*. 1986;232:506-508.
19. Lopez AF, Nicola NA, Burgess AW, et al. Activation of granulocyte cytotoxic function by purified mouse colony-stimulating factors. *J Immunol*. 1983;131:2983-2988.
20. Kurland JI, Pelus LM, Ralph P, et al. Induction of prostaglandin E synthesis in normal and neoplastic macrophages: role for colony-stimulating factor(s) distinct from effects on myeloid progenitor cell proliferation. *Proc Natl Acad Sci USA*. 1979;76:2326-2330.
21. Hamilton JA, Stanley ER, Burgess AW, et al. Stimulation of macrophage plasminogen activator activity by colony-stimulating factors. *J Cell Physiol*. 1980;103:435-445.
22. Weisbart RH, Kwan L, Golde DW, et al. Human GM-CSF primes neutrophils for enhanced oxidative metabolism in response to the major physiological chemotactic agents. *Blood*. 1987;69:18-21.
23. Lopez AF, Williamson DJ, Gamble JR, et al. Recombinant human granulocyte-macrophage colony-stimulating factor stimulates *in vitro* mature human neutrophil and eosinophil function, surface receptor expression, and survival. *J Clin Invest*. 1986;78:1220-1228.
24. Peterson V, Hansborough J, Buerk C, et al. Regulation of granulopoiesis following severe thermal injury. *J Trauma*. 1983;23:19-24.
25. Iyengar VG, Peterson VM, Rundus C, et al. Postburn serum inhibits in

vitro production of colony-stimulating factor by mononuclear peripheral blood cells. *Int J Cell Cloning*. 1986;4:472-482.

26. Allen RC, Pruitt BA Jr. Humoral-phagocyte axis of immune defense in burn patients. *Arch Surg*. 1982;117:133-140.

27. Peterson VM, Robinson WA, Wallner SF, et al. Granulocyte stem cells are decreased in humans with fatal burns. *J Trauma*. 1985;25:413-418.

28. Andreeff M, Welte K. Hematopoietic colony-stimulating factors. *Semin Oncol*. 1989;16:211-220.

29. Donahue RE, Wang EA, Stone DK, et al. Stimulation of hematopoiesis in primates by continuous infusion of human GM-CSF. *Nature*. 1986;321:872-875.

30. Gasson JC, Weisbart RH, Kaufman SE, et al. Purified human granulocyte-macrophage colony-stimulating factor: direct action on neutrophils. *Science*. 1984;226:1339-1342.

31. Vadas MA, Nicola NA, Metcalf D. Activation of antibody-dependent cell-mediated cytotoxicity of human neutrophils and eosinophils by separate colony-stimulating factors. *J Immunol*. 1983;130:795-799.

32. Vadhan-Raj S, Buescher S, Broxmeyer, et al. Stimulation of myelopoiesis in patients with aplastic anemia by recombinant human granulocyte-macrophage colony-stimulating factor. *N Engl J Med*. 1988;319:1620-1634.

33. Groopman JE, Mitsuyasu RT, DeLeo MJ, et al. Effect of recombinant human granulocyte-macrophage colony-stimulating factor on myelopoiesis in the acquired immunodeficiency syndrome. *N Engl J Med*. 1987;317:593-598.

34. Jakubowski AA, Souza L, Kelly F, et al. Effects of human granulocyte colony-stimulating factor in a patient with idiopathic neutropenia. *N Engl J Med*. 1989;320:38-42.

35. Antman KS, Griffin JD, Elias A, et al. Effect of recombinant human granulocyte-macrophage colony-stimulating factor on chemotherapy-induced myelosuppression. *N Engl J Med*. 1988;319:593-598.

36. Gabilove JL, Jakubowski A, Scher H, et al. Effect on granulocyte colony-stimulating factor on neutropenia and associated morbidity due to chemotherapy for transitional-cell carcinoma on the urothelium. *N Engl J Med*. 1988;318:1414-1422.

37. Brandt SJ, Peters WP, Atwater SK, et al. Effect of recombinant human granulocyte-macrophage colony-stimulating factor on hematopoietic reconstitution after high-dose chemotherapy and autologous bone marrow transplantation. *N Engl J Med*. 1988;318:869-876.

38. Weisbart RH, Kwan L, Golde DW, et al. Human GM-CSF primes neutrophils for enhanced oxidative metabolism in response to the major physiological chemoattractants. *Blood*. 1987;69:18-21.

39. Weisbart RH, Golde DW, Clark SC, et al. Human granulocyte-macrophage colony-stimulating factor is a neutrophil activator. *Nature*. 1985;314:361-363.

40. Cioffi WG, Burleson DG, Jordan BS, et al. Granulocyte function following thermal injury. Read before the 22nd Annual Meeting of the American Burn Association, March 29, 1990, Las Vegas, Nev.

## Discussion

CORA K. OGLE, MD, Cincinnati, Ohio: The total burn surface area of the study patients ranged from 20% to 70%, and the patients with larger burns developed complications. What will the increase of superoxide release with GM-CSF? Was the result of improvement of release from a defective cell or was the improvement the result of new cells released from the bone marrow?

DAVID H. LIVINGSTON, MD, Newark, NJ: How was the dosage of GM-CSF derived and by what route was it delivered?

JONATHAN MEAKINS, MD, Montreal, Quebec: Was there any clinical effect as a result of the administration of GM-CSF?

RONALD V. MAIER, MD, Seattle, Wash: If administration of GM-CSF stimulates the release of very immature cells, are the changes that are monitored basically the functional changes of an aging neutrophil?

H. DAVID REINES, MD, Richmond, Va: What about the maturity of the cells and the differential cell counts? Do a large number of band cells appear in the treated patients?

DR CIOFFI: Originally, patients with burns of 20% to 40% were enrolled into the study and then the population of patients was expanded to those with burns of 40% to 70% after it was established that marked adverse effects were not seen in the small burns. Normal granulocytes have been incubated in vitro with GM-CSF and have shown a progression of priming of the neutrophil, but this has not been done for neutrophils of burn patients. The intravenous doses of 3 to 10  $\mu$ g/kg were derived from primate studies with the thought that it would double the white blood cell counts. In regard to the clinical effects of the compound, two of the treated patients developed abscesses. The GM-CSF shortens the time that the white blood cell spends in the bone marrow, so we may just be looking at younger cells that have more myeloperoxidase activity. However, a lot of younger cell types were not seen in the peripheral blood. The only alteration was that the patients developed a relative granulocytopenia after the cessation of GM-CSF administration for approximately 1 week, which subsequently returned to normal.

Accession For	
NTIS CRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By	
Distribution /	
Availability Codes	
Dist	Avail and/or Special
A-1	20

